second is the GAP program, available as part of the Wisconsin Genetics Software Package, that uses the algorithm of Needleman and Wunsch (*J. Mol. Biol.* 48:443-453, 1970) to find the alignment of two complete sequences that maximizes the number of matches and minimizes the number of gaps. Default gap creation penalty values and gap extension penalty values in Version 10 of the Wisconsin Genetics Software Package for nucleotide sequences are 50 and 3, respectively, and for protein sequences are 8 and 2, respectively. Unless otherwise specified, references to the GAP program or algorithm refer to the GAP program or algorithm in version 10 of the Wisconsin Genetics Software Package. The gap creation and gap extension penalties can be expressed as an integer selected from the group of integers consisting of from 0 to 200. Thus, for example, the gap creation and gap extension penalties can be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 or greater. The scoring matrix used in Version 10 of the Wisconsin Genetics Software Package is BLOSUM62 (*see* Henikoff & Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915).

Please replace the paragraph beginning at page 32, line 6, with the following rewritten paragraph:

PCC 6803 was used as a tool for identification of genes encoding enzymes involved in biosynthesis of tocopherols. *Synechocystis* is a cyanobacterium capable of tocopherol biosynthesis. The entire genome of this photosynthetic organism has been recently sequenced (Kaneko et al., 1996) and the data is available on a public searchable database, called CyanoBase. Using CyanoBase, we have identified an open reading frame (SLR1736) encoding a phytyl/prenyltransferase involved in the biosynthesis of 2-methyl-6-phytylplastoquinol, one of the tocopherol precursors. This open reading frame was identified based on similarity with the phytyl/prenyltransferase SLR0056, a phytyl/prenyltransferase involved in the biosynthesis of chlorophyll in *Synechocystis* PCC 6803. SLR0056 exhibits a high

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homology with the previously identified chlorophyllide/phytyl/prenyltransferases from many cyanobacteria and *A. thaliana* (Lopez et al., 1996), suggesting that this enzyme is also involved in chlorophyll synthesis. --

Please replace the paragraph beginning at page 33, line 28, with the following rewritten paragraph:

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--Chromosomal DNA from wild type Synechocystis PCC 6803 was isolated according to Williams (Methods in Enzymology (1987) 167:766-778). The primers represented by SEQ ID NOS: 7 (SLR1736F) and 8 (SLR1736R) were designed using Mac Vector computer program to amplify a 1.022 kb fragment containing the SLR1736 open reading frame. NdeI and BamHI sites were added to the primers to facilitate sub-cloning for expression purposes. ATG in the SLR1736F primer is the start codon for the SLR1736 open reading frame published in the CyanoBase Web-site. Taq polymerase (Gibco BRL) was used for gene disruption purposes and later Vent polymerase (NEB) was used for expression purposes following the manufacturer's recommendations. The following cycles were performed:
For Taq polymerase amplification:
95°C/5 minutes (1 cycle)
95°C/45 seconds, 45°C/45 seconds, 68°C/45 seconds (5 cycles)
95°C/45 seconds, 52°C/45 seconds, 72°C/45 seconds (30 cycles)
72°C/10 minutes
The same thermocycler conditions were used to amplify SLR1736 with Vent polymerase except that elongation times were extended to 2 minutes. --

Please replace the paragraph beginning at page 39, line 15, with the following rewritten paragraph:

-- A developing seed-specific cDNA library from A.thaliana (lambda-ZAP type, provided by John Ohlrogge at the Michigan State University) was screened using a PCR product from wild type A. thaliana genomic DNA (Ler ecotype) which exhibits a high degree of homology with the Synechocystis phytyl transferase. Primers represented by SEQ ID NO: 5 (AT1736F) and SEQ ID NO: 6 (AT1736B) were used to amplify about 1kb fragment corresponding to 60238 – 61229 bp region of the BAC clone F19F24 (A. thaliana database at Stanford). The following program was used to amplify this fragment with Vent DNA polymerase (New England Biolabs):
95°C/5 minutes (1 cycle)
95°C/45 seconds; 50°C/45 seconds, 72°C/1 minute (30 cycles)
72°C/10 minutes (1 cycle). --

B5

Please replace the paragraph beginning at page 39, line 27, (final paragraph) with the following rewritten paragraph:

-- The PCR product was then sub-cloned into *Eco*RV site of pBluescript KS (Stratagene) as in the case of the cyanobacterial phytyl transferase presented above and sequenced from both ends using T3 and T7 primers (Stratagene) to ensure the identity of the sub-cloned fragment. A 300 bp fragment of the insert (5'-end) was released with *Eco*RI from the vector and used as a radioactively-labeled probe to obtain full-length clones. About 2.5 million plaques of the seed-specific library were screened using standard procedures (Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989). Molecular Cloning. 2nd edition, Cold Spring Harbor Laboratory Press). 16 positive non-purified plaques were chosen for PCR analysis using T3 and SEQ ID NO: 30 (AT1736T7c) which is an internal primer for the phytyl transferase. Clones #1, 3, 5, 8, 11, 12, and 14 were selected for further purification and single clone excision, performed according to manufacturer (Stratagene), to obtain individual clones in pBluescript SK plasmids. Each clone was sequenced from each end using T3 and T7 primer. The longest clone, #11 – about 1.6 kb, was chosen for complete

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sequencing which is in progress now. All clones were aligned to the genomic clone F19F24 from *A. thaliana* to confirm their identity, identify introns and find possible sequencing mistakes in the genomic sequence. We believe that ATG codon (59220 bp on F19F24) is the start codon of the phytyl transferase involved in tocopherol synthesis in *A. thaliana*. Starting from this methionine, the first 36 amino acids represent the chloroplast thylakoid membrane-targeting sequence. --

B6

Please replace the paragraph beginning at page 43, line 25, (final paragraph) with the following rewritten paragraph:

-- Colony hybridization is conducted as described by Sambrook, J., Fritsch, E.F. and Maniatis, T., (in Molecular Cloning: A laboratory Manual, 2nd Edition). The following probes were used in colony hybridization:

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1. First strand cDNA from the same tissue as the library was made to remove the most redundant clones.
2. 48-192 most redundant cDNA clones from the same library based on previous sequencing data.
3. 192 most redundant cDNA clones in the entire corn sequence database.
4. A Sal-A20 oligo nucleotide removes clones containing a poly A tail but no cDNA.
5. cDNA clones derived from rRNA. --

Please replace the paragraph beginning at page 44, line 8, with the following rewritten paragraph:

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-- Gene identities were determined by conducting BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al., (1993) *J. Mol. Biol.* 215:403-410; searches under default parameters for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank,

the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish, W. and States, D. J. *Nature Genetics* 3:266-272 (1993)) provided by the NCBI. In some cases, the sequencing data from two or more clones containing overlapping segments of DNA were used to construct contiguous DNA sequences. --

B8

Please replace the paragraph beginning at page 55, line 15, with the following rewritten paragraph:

-- The ability to change the levels of total tocopherol levels in plants by transforming them with sequences encoding the maize phytyl/prenyltransferase was tested by preparing transgenic soybean somatic embryos and assaying the tocopherol and oil levels. Plasmid DNA from clone poo18chste82r was used as a template for the amplification of the open reading from pcr by using the primers represented by SEQ ID NO: 31 (forward primer) and SEQ ID NO: 32 (reverse primer). Pfu polymerase was used according to the manufacturers recommendations (Stratagene). The following pcr reaction mix contained the following: 5ng plasmid, 25nmoles dNTPs, 5% DMSO, 1x pcr buffer (supplied), 30nmoles primers, 5U pfu polymerase in 100ul reaction volume. The pcr reaction conditions were as follows Step 1, 45s 94°C; step 2 25 cycles of 45s 94°C, 45s 58°C annealing, 2min extension 72°C. Step 3 72°C 10min, step 4 0°C. The pcr product was purified by agarose gel electrophoresis (!% agarose in TAE), the ethidium bromide visualized band cut out and purified from the gel by using a QIAquick Gel Extraction kit (Qiagen) according to the manufacturers recommendations. The

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